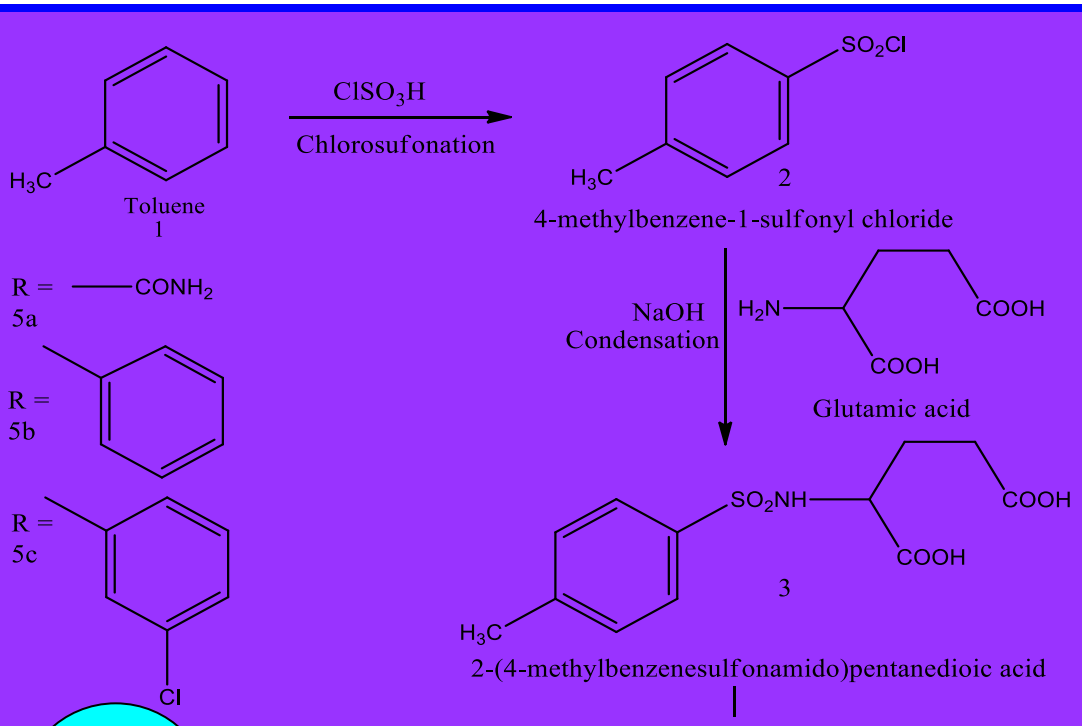

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Biological activity of some novel synthesized 2-(4-methylbenzene-sulphonamido)pentanedioic acid bis amide derivatives: *In vitro* and *in vivo* antineoplastic activity

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Abstract

In the present work few novel 2-(4-methylbenzenesulphonamido) pentanedioic acid bis amide derivatives and the basic compound 2-(4-methylphenyl-sulfonamido)pentanedioic acid have been synthesized, characterized and screened for their possible antineoplastic activity both *in vitro* and *in vivo*. The *in vitro* activity was performed against five human cell lines like human breast cancer (MCF-7), leukemia (K-562), ovarian cancer (OVACAR-3), human colon adenocarcinoma (HT-29) and human kidney carcinoma (A-498). The *in vivo* activity was performed in female swiss albino mice against *Ehrlich ascites carcinoma* (EAC). Among the synthesized compounds, ureide, anilide, *p*-nitoanilide and *o*-bromoanilide derivatives of 2 -(4-methyl benzene sulphonyl)-pentanedioic acid bis amides showed encouraging activity in both the *in vitro* and *in vivo* compared to other compounds.

Introduction

Cancer is a class of disease in which group of cells display uncontrolled growth, invasion and sometimes metastasis. Cancer affects people at all ages with the risk for most types increasing with age (Cancer Research UK, 2007). Cancer caused about 13% of all human deaths in 2007 (7.6 million) (WHO, 2006; American Cancer Society, 2007).

Glutamic acid (2-amino pentanedioic acid) plays an important role in the biosynthesis of purine and pyrimidine bases of DNA and RNA (Rodwell, 2000). It is metabolized to L-glutamine by L-glutamine synthetase and this metabolic process is essential for normal maintenance of cells. The synthesis of L-glutamine is hindered in neoplastic cells due to lower reactivity of L-glutamine synthetase. Thus antagonists of this enzyme can interfere with the metabolic role of L-glutamine and act as anti-cancer agents (Hartman, 1970). Azaserine and 6-diaza-5-oxo-L-norleucine antagonized the metabolic process involving L-glutamine and exhibited antitumor activity in animal models (Eidinoff, 1958). The importance of non-essential amino acid glutamine in proliferation of human tumor cells was studied exten -sively (Graff et al., 1940; Petit, 1977). All tumor cells studied were found to have a high activity of phosphate -dependent glutaminase utilizing glutamine from the medium during long-term culture (Graff et al., 1940). Beside glucose, glutamine is assumed to be the main energy source in tumor cells (Keren and Stark, 1988). It also plays a central role in multiple metabolic pathways and considered to be the most essential component of tissue/cell culture media (Sugita, 1995) for not only as the nitrogen source but also as the carbon source. After a definite time interval, all cells start mutation in cell culture medium, which is also indicative for the role of

glutamine in cancer (Rosowsky et al., 1979). Aryl sulphatase C (ASC) family of transporters is involved in the mediation of glutamine uptake and glutamine, in the form of glutamate, and cysteine are supplied perhaps for glutathione synthesis (Debnath et al., 2002). Thus, the structural variants of glutamine attracted our attention to develop possible anti-cancer agents, which may act through glutamine and/or folic acid antagonism.

Materials and Methods

Commercially available reagents and starting materials for the synthesis were obtained from E. Merck, India, CDH, s.d. Fine Chem, India and Qualigens, India. Silica gel G used for TLC was obtained from E. Merck. The reaction was monitored by TLC using on 0.25 mm E. Merck silica gel $60F_{252}$ precoated plates, which were visualized under UV light. Melting points were determined in an open glass capillary using a Kjeldahl flask containing paraffin and are uncorrected. The proton and carbon magnetic resonance spectra (^{1}H NMR, ^{13}C NMR) were recorded on a Bruker 400 MHz instrument (Bruker, Germany) in dimethyl sulfoxide-d_6 (DMSO-d_6) using tetramethylsilane as internal standard. Chemical shifts (d) are expressed in ppm and coupling constants (s) singlet, (d) doublet, (t) triplet, (m) multiplet. Position of carbons described in ^{13}C NMR interpretation is as per general structure (Figure 1) for all the compounds except *Va*. The infrared spectra of compounds were recorded in KBr on Fourier Transform (FTIR-8400S, Shimadzu, Japan) infrared spectrophotometer. Mass spectra (FAB) were recorded on LC-MS/MS (API-4000 TM, Applied BioSystems, MDS SCIEX, Canada). Elemental analyses were performed on a Perkin-Elmer model 240c analyzer (Perkin Elmer, USA).

General procedure for synthesis of 2-(4-methyl-benzenesulfo-namido)pentanedioic acid bis amide derivatives (5a-l)

2-(4-Methylbenzenesulfonamido)pentanedioic acid bis amide derivatives were synthesized based on following procedure.

Synthesis of 4-methylbenzene-1-sulfonyl chloride (2)

Tolu-ene (9.2 mL, 0.1 mol) was taken in a 250 mL three necked round bottom flask placed on an ice bath fitted with a mercury sealed mechanical stirrer, a calcium chloride guard tube and a 100 mL dropping funnel. Chlorosulfonic acid (25 mL, 3 equiv., 0.36 mol) was placed in the dropping funnel and was added drop wise slowly by continuous stirring. The temperature of the ice bath was maintained at 0°C. After the addition of the chlorosulfonic acid completely the mixture was allowed to come to room temperature. Then the product was poured slowly into well stirred crushed ice. The crude 4-methylbenzene-1-sulfonyl chloride (**2**) was filtered and washed with water to remove the excess acid. The crude product was recrystalized from water. The yield was 15.2 g (84.2%) and m.p.: 155-57°C.

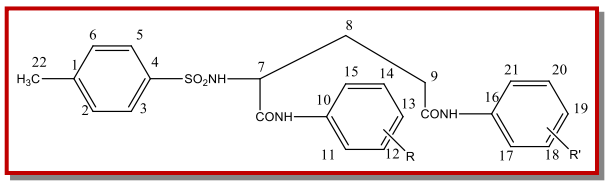


Figure 1: General structure for 2-(4-methyl-benzenesulphonamido) pentanedioic acid bis amides

Synthesis of 2-(4-methylbenzenesulfonamido)pentanedioic acid (3)

This was prepared form 4-methylbenzene-1-sulfonyl chloride (**2**) and L-(+)-glutamic acid according to the procedure reported below.

L-(+)-glutamic acid (20 g, 0.1 mole) was taken in a 250 mL conical flask and placed on a water both, fitted with a magnetic stirrer. Sodium hydroxide solution (2N) was added slowly till all the L-glutamic acid dissolved and the mass became distinctly alkaline to phenolphthalein. The water bath was maintained at 60-80°C and 4-methylbenzene-1-sulfonyl chloride (**2**; 36.1 g, 0.2 mol) was added slowly with continuous stirring and simultaneous addition of sodium hydroxide solution (2N) to keep the mass alkaline. The reaction was continued till a clear homogeneous solution results. After the reaction was over, it was allowed to cool, acidified to congored with concentrated hydrochloric acid, saturated with sodium chloride, extracted with chloroform, and allowed to dry overnight with anhydrous magnesium sulfate. Chloroform was removed to yield the crude 2-(4-methylbenzenesulfonamido)pentanedioic acid (**3**). The crude product was recrystalized from hot water after charcoal treatment. Yield 57.9%, m.p.: 150-53°C. R_f 0.76. Neutral equivalent (found: 297.20, Calc. For $C_{12}H_{15}NO_6S$: 301). IR (KBr) υ_{max} (cm^{-1}) 3136.95 (C-H str. of phenyl ring), 3059.14-2620.21 (O-H str. of COOH), 1667.16, (C=O str.), 1502.34 (C=C str. of phenyl ring), 1322.65 (C-O str. or O-H def of COOH), 1381.06 (S=O str. antisym of SO_2N), 1267.80 (S=O str. sym of SO_2N), 825 (out of plane C-H def due to p-subs in phenyl ring), 3294.93 (N-H str.), 1664.16 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.519 (d, 2H, 2', 6' H of C_6H_4-CH_3), 7.071 (d, 2H, 3', 5' H of C_6H_4-CH_3), 3.7 (t, 3H, -OC-C.H-CH_2-), 2.152 (s, 1H, -CH_3), 2.14 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ = 129.12 (C-1 & 5), 118.14 (C-2 & 4), 143.76 (C-3), 168.32 (C-8), 176.20 (C-18). MS (FAB; m/z): 301. [M^{+1}]. Elemental analysis ($C_{12}H_{15}NO_6S$); calcd. C, 47.83; H, 5.02; N, 4.65; found C, 47.70; H, 4.91; N, 4.59%.

Synthesis of 2-(4-methylbenzenesulfonamido)pentane-dioyl dichloride (4)

2-(4-Methylbenzenesulfonamido)pentane-dioic acid (**3**;

3.01 g, 0.01 mol) was taken in a 50 mL round bottom flask fitted with a reflux condenser and a calcium chloride guard tube. Thionyl chloride (5 mL) was added to it and refluxed in a stem bath for 2 hours. The excess thionyl chloride was removed by distillation and dry benzene (10 mL) was used to remove hydro-chloric acid fumes by distillation. This product obtained was 2-(4-methylbenzenesulfonamido)pentanedioyl dichloride (**4**). This was later used in the subsequent steps. Yield: 2.80 g (82.84%); m.p.: 105-07°C.

Synthesis of 2-(4-methylbenzenesulfonamido)pentanedioic acid bis amide derivatives (5a-l)

The 2-(4-methylbenzene-sulfonamido)pentanedioyl dichloride (**4**) formed was dissolved in dry benzene (10 mL) and the whole mass was cooled by dipping in ice water. Aniline and its derivatives, previously cooled was added to it and mixed well. The whole mass was transferred in a mortar and pestle and triturated when the product was obtained. It was then acidified with dilute hydrochloric acid. The precipitate obtained was filtered and washed with distilled water to remove excess acid. The residue was dried and recrystallized from dilute ethanol after charcoal treatment.

[5-(Carbomoylamino)-4-(4-methylbenzenesulfonamido) -5-oxopentanoyl]urea (5a)

Greyish colour solid; yield: 41.53%; m.p.: 183-186°C; R_f 0.87; IR (KBr) υ_{max} (cm^{-1}): 2966.54-2754.28 (O-H str. of COOH), 1651.5 (C=O str.), 1547.21 (C=C str. of phenyl ring), 1328.27 (S=O str. antisym of SO_2N), 1310.18 (S=O str. sym of SO_2N), 860.61 (out of plane C-H def due to p -subs in phenyl ring), 3375 (N-H str.), 1621 (N-H bending). ^{1}H NMR (DMSO-d_6): δ 7.45(d, 2H, 2', 6' H of C_6H_4-CH_3), 7.312 (d, 2H, 3', 5' H of C_6H_4-CH_3), 4.042 (t, 3H, -OC-C.H-CH_2-), 2.604(s, 1H, -CH_3), 2.203 (s, 1H, -SO_2NH).^{13}C NMR (DMSO-d_6): δ 141.98 (C-1), 128.54 (C-2 & 6), 126.20 (C-3 & 5), 139.50 (C-4), 58.34 (C-7), 27.21 (C-8), 36.15 (C-9), 154.60 (C-11), 156.38 (C-14), 21.62 (C-10). MS (FAB; *m/z*): 385. [M^{+1}]. Elemental analysis ($C_{14}H_{19}N_5O_6S$); calcd. C, 43.63; H, 4.97; N, 18.17; found C, 43.53; H, 5.05; N, 18.19%.

2-(4-Methylbenzenesulphonamido)-N,N′-(diphenyl) pentane-diamide (5b)

White color solid; yield: 88.45%; m.p.: 206-208°C; R_f 0.68; IR (KBr) υ_{max} (cm^{-1}): 2926.24-2653.68 (O-H str. of COOH), 1681.8 (C=O str.), 1577.31 (C=C str. of phenyl ring), 1308.3 (S=O str. antisym of SO_2N), 1220.28 (S=O str. sym of SO_2N), 860.61 (out of plane C-H def due to p -subs in phenyl ring), 3397 (N-H str.), 1630 (N-H bending).^{1}H NMR (DMSO-d_6): δ 7.547 (d, 2H, 2', 6' H of C_6H_4-CH_3), 7.341 (d, 2H, 3', 5' H of C_6H_4-CH_3), 3.8 (t, 3H, -OC-C.H-CH_2-), 2.551(s, 1H, -CH_3), 2.12 (s, 1H, -SO_2NH-).^{13}C NMR (DMSO-d_6): δ 140.81 (C-1), 128.44 (C -2 & 6), 128.21 (C-3 & 5), 137.22 (C-4), 57.51 (C-7), 27.00 (C-8), 36.32 (C-9), 120.73 (C-11& 15), 128.78 (C-12 &14), 122.66 (C-17 & 21), 128.58 (C-18 & 20), 21.43 (C-22). MS (FAB; *m/z*): 451. [M^{+1}]. Elemental analysis ($C_{24}H_{23}N_3O_4S$); calcd. C, 63.85; H, 5.09; N, 9.31; found C, 55.20; H, 5.15; N, 9.19%.

2-(4-Methylbenzenesulphonamido)-N,N′-bis(3-chlorophenyl)pentanediamide (5c)

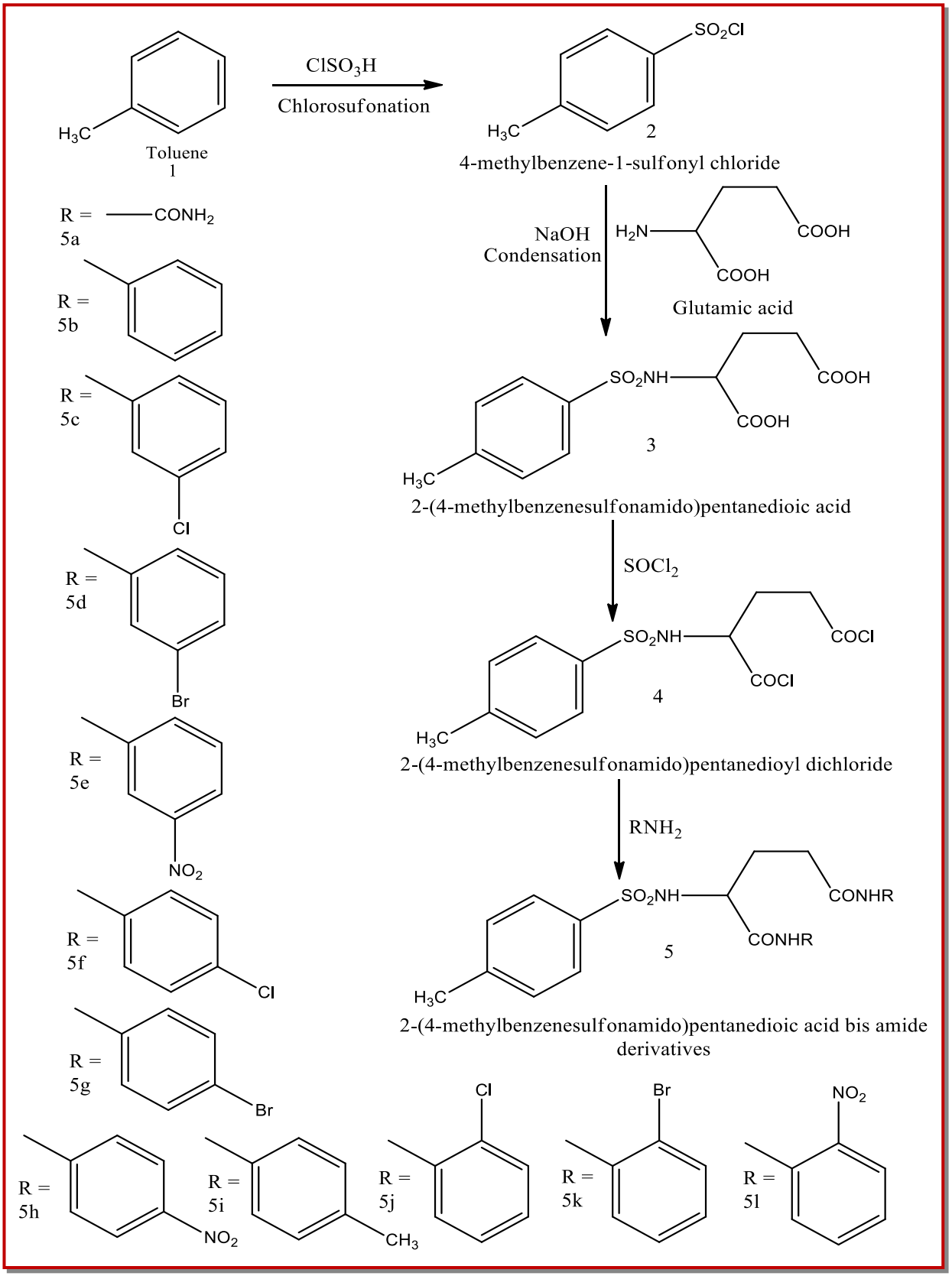
White colour solid; yield: 53.64%; m.p.: 154-157°C; R_f 0.82; IR (KBr) υ_{max} (cm^{-1}): 2807-2542 (O-H str. of COOH), 1671.47 (C=O str.), 1507.64 (C=C str. of phenyl ring), 1348 (C-N phenyl ring), 1304.15 (S=O str. antisym of SO_2N), 1210.7 (S=O str. sym of SO_2N), 865 (out of plane C-H def due to p-subs in phenyl ring), 710.6 (C-Clstr. of phenyl ring), 3433.21 (N-H str.), 1593 (N-H bending).^{1}H NMR (DMSO-d_6): δ 7.4251(d, 2H, 2', 6' H of C_6H_4-CH_3), 7.304 (d, 2H, 3', 5' H of C_6H_4-CH_3), 7.271(d, 2H, 2', 6' H of C_6H_4-Cl), 7.201 (d, 2H, 3', 5' H of C_6H_4-Cl), 4.0 (t, 3H, -OC-C.H-CH_2-), 2.507(s, 1H, -CH_3), 2.40 (q, 4H,-H_2C-H_2C -), 2.004 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6): δ 141.26 (C-1), 126.54 (C-2 & 6), 129.11 (C-3 & 5), 136.87 (C-4), 57.51 (C-7), 27.42 (C-8), 34.27 (C-9), 121.36 (C-11), 130.43 (C-12), 122.32 (C-14), 122.41 (C-15), 118.95 (C-17), 130.23 (C-18), 127.34 (C-19), 120.14 (C-20), 21.30 (C-22). MS (FAB; *m/z*): 520. [M^{+1}]. Elemental analysis ($C_{24}H_{23}N_3O_4SCl_2$); calcd. C, 55.38; H, 4.42; N, 8.07; found C, 53.14; H, 4.61; N, 8.09%.

2-(4-Methylbenzenesulphonamido)-N,N′-bis(3-bromophenyl)pentanediamide (5d)

Yellow colour solid; yield: 66.76%; m.p.: 192-194°C; R_f 0.66; IR (KBr) υ_{max} (cm^{-1}): 3012.14-2753.38 (O-H str. of COOH), 1651.6 (C=O str.), 1554.11 (C=C str. of phenyl ring), 1328 (S=O str. antisym of SO_2N), 1234.18 (S=O str. sym of SO_2N), 890.61 (out of plane C-H defdue to p-subs in phenyl ring), 3567 (N-H str.), 1641 (N-H bending).^{1}H NMR (DMSO-d_6): δ 7.522 (d, 2H, 2', 6' H of C_6H_4-CH_3), 7.621 (d, 2H, 3', 5' H of C_6H_4-CH_3), 7.824 (d, 2H, 2', 6' H of C_6H_4-Br), 7.98 (d, 2H, 3', 5' H of C_6H_4-Br), 3.4 (t, 3H, -OC-C.H-CH_2-), 2.42 (s, 1H, -CH_3), 2.0 (s, 1H, -SO_2NH-).^{13}C NMR (DMSO-d_6): δ 141.12 (C-1), 126.24 (C-2 & 6), 127.15 (C-3 & 5), 137.32 (C-4), 58.41 (C-7), 26.72 (C-8), 37.29 (C-9), 120.36 (C-11), 130.66 (C-12), 121.74 (C-14), 124.24 (C-15), 119.95 (C-17), 130.66 (C-18), 126.67 (C-19), 121.79 (C-20), 21.43 (C-22).MS (FAB; *m/z*): 609. [M^{+1}]. Elemental analysis ($C_{24}H_{23}N_3O_4SBr_2$); calcd. C, 47.29; H, 3.77; N, 6.89; found C, 47.23; H, 3.59; N, 6.80%.

2-(4-Methylbenzenesulphonamido)-N,N′-bis(3-nitrophenyl)pentanediamide (5e)

White colour solid; yield: 64.71%; m.p.: 176-178°C; R_f 0.61; IR (KBr) υ_{max} (cm^{-1}): 2858.9-2574.55 (O-H str. of COOH), 1696.07 (C=O str.), 1524.49 (C=C str. of phenyl ring), 1280.84 (S=O str. antisym of SO_2N), 1210.6 (S=O str. sym of SO_2N), 878.49 (out of plane C-H def due to p -subs in phenyl ring), 729.04 (C-Clstr. of phenyl ring),1582.26 (C-NO_2), 3500 (N-H str.), 1617 (N-H



Scheme 1: Synthesis of 2-(4-methylbenzenesulfonamido) pentanedioic acid bis amide derivatives

bending). ^{1}H NMR (DMSO-d_6): δ 7.60(d, 2H, 2', 6' H of C_6H_4-CH_3), 7.55 (d, 2H, 3', 5' H of C_6H_4-CH_3), 7.99(d, 2H, 2', 6' H of C_6H_4-NO_2), 7.92 (d, 2H, 3', 5' H of C_6H_4-NO_2), 3.5 (t, 3H, -OC-C.H-CH_2-), 2.507(s, 1H, -CH_3), 2.0 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6): δ 142.32 (C-1), 127.43 (C-2 & 6), 126.52 (C-3 & 5), 135.24 (C-4), 57.21 (C-7), 24.21 (C-8), 37.43 (C-9), 122.30 (C-11), 131.26 (C-12), 120.14 (C-14), 125.27 (C-15), 118.57 (C-17), 131.31 (C-18), 127.67 (C-19), 121.85 (C-20), 22.31 (C-22).MS (FAB; *m/z*): 541. [M^{+1}]. Elemental analysis ($C_{24}H_{23}N_5O_8S$); calcd. C, 53.23; H, 4.25; N, 12.93; found C, 53.08; H, 4.11; N, 12.79%.

2-(4-Methylbenzenesulphonamido)-N,N′-bis(4-chlorophenyl)pentanediamide (5f)

White colour solid; yield: 75.87%; m.p.: 109-111°C; R_f 0.84; IR (KBr) υ_{max} (cm^{-1}): 2930.24-2553.68 (O-H str. of COOH), 1672 (C=O str.), 1551.33 (C=C str. of phenyl ring), 1342.22 (S=O str. antisym of SO_2N), 1214.25 (S=O str. sym of SO_2N), 848.13 (out of plane C-H defdue to p-subs in phenyl ring), 3295 (N-H str.), 1645(N-H bending). ^{1}H NMR (DMSO-d_6): δ 7.3821(d, 2H, 2', 6' H of C_6H_4-CH_3), 7.291 (d, 2H, 3', 5' H of C_6H_4-CH_3), 7.252 (d, 2H, 2', 6' H of C_6H_4-Cl), 7.243 (d, 2H, 3', 5' H of C_6H_4-Cl), 4.1 (t, 3H, -OC-C.H-CH_2-), 2.417(s, 1H, -CH_3), 2.42 (q, 4H,-H_2C-H_2C-), 2.041 (s, 1H, -SO_2NH-).^{13}C NMR (DMSO-d_6): δ 142.42 (C-1), 129.24 (C-2 & 6), 126.21 (C-3 & 5), 136.43 (C-4), 55.31 (C-7), 27.32 (C-8), 35.32 (C-9), 122.41 (C-11 & 15), 128.47 (C-12 & 14), 121.41 (C-17 & 21), 129.14 (C-18 & 20), 22.03 (C-22). MS (FAB; *m/z*): 520. [M^{+1}]. Elemental analysis ($C_{24}H_{23}N_3O_4SCl_2$); calcd. C, 55.38; H, 4.42; N, 8.07; found C, 55.28; H, 4.54; N, 8.13%.

2-(4-methylbenzenesulphonamido)-N,N′-bis(4-bromophenyl)pentanediamide (5g)

White colour solid; yield: 53.45%; m.p.: 140-141°C; R_f 0.78; IR (KBr) υ_{max} (cm^{-1}): 3012.54-2753.66 (O-H str. of COOH), 1570.8 (C=O str.), 1527.31 (C=C str. of phenyl ring), 1354.5 (S=O str. antisym of SO_2N), 1260.48 (S=O str. sym of SO_2N), 865.21 (out of plane C-H def due to p-subs in phenyl ring), 3287.22 (N-H str.), 1621 (N-H bending).^{1}H NMR (DMSO-d_6): δ 7.741 (d, 2H, 2', 6' H of C_6H_4-CH_3), 7.542 (d, 2H, 3', 5' H of C_6H_4-CH_3), 7.841 (d, 2H, 2', 6' H of C_6H_4-Br), 7.944 (d, 2H, 3', 5' H of C_6H_4-Br), 3.54 (t, 3H, -OC-C.H-CH_2-), 2.421 (s, 1H, -CH_3), 2.0 (s, 1H, -SO_2NH-).^{13}C NMR (DMSO-d_6): δ 140.16 (C-1), 130.42 (C-2 & 6), 127.43 (C-3 & 5), 137.43 (C-4), 56.43 (C-7), 27.65 (C-8), 35.21 (C-9), 121.32 (C-11 & 15), 129.37 (C-12 & 14), 121.75 (C-17 & 21), 129.54 (C-18 & 20), 21.43 (C-22).MS (FAB; *m/z*): 609.[M^{+1}]. Elemental analysis ($C_{24}H_{23}N_3O_4SBr_2$); calcd. C, 47.29; H, 3.77; N, 6.89; found C, 47.37; H, 3.67; N, 6.78%.

2-(4-Methylbenzenesulphonamido)-N,N′-bis(4-nitrophenyl)pentanediamide (5h)

White colour solid; yield: 54.34%; m.p.: 164-166°C; R_f 0.91; IR (KBr) υ_{max} (cm^{-1}): 2946.54-2643.68 (O-H str. of COOH), 1641.8 (C=O str.), 1574.34 (C=C str. of phenyl ring), 1312.13 (S=O str. antisym of SO_2N), 1232.18 (S=O str. sym of SO_2N), 872.51 (out of plane C-H def due to p-subs in phenyl ring), 3395 (N-H str.), 1628 (N-H bending).^{1}H NMR (DMSO-d_6): δ 7.621 (d, 2H, 2', 6' H of C_6H_4-CH_3), 7.578 (d, 2H, 3', 5' H of C_6H_4-CH_3), 7.892 (d, 2H, 2', 6' H of C_6H_4-NO_2), 7.95 (d, 2H, 3', 5' H of C_6H_4-NO_2), 3.6 (t, 3H, -OC-C.H-CH_2-), 2.52 (s, 1H, -CH_3), 2.1 (s, 1H, -SO_2NH-).^{13}C NMR (DMSO-d_6): δ 139.18 (C-1), 126.54 (C-2 & 6), 129.11 (C-3 & 5), 136.43 (C-4), 58.17 (C-7), 28.24 (C-8), 37.24 (C-9), 121.83 (C-11 & 15), 129.67 (C-12 & 14), 123.26 (C-17 & 21), 128.57 (C-18 & 20), 20.12(C-22).MS (FAB; *m/z*): 541. [M^{+1}]. Elemental analysis ($C_{24}H_{23}N_5O_8S$); calcd. C, 53.23; H, 4.25; N, 12.93; found C, 53.13; H, 4.21; N, 13.03%.

2-(4-Methylbenzenesulphonamido)-N,N′-bis(4-methylphenyl)pentanediamide (5i)

White colour solid; yield: 43.76%; m.p.: 198-200°C; R_f 0.74; IR (KBr) υ_{max} (cm^{-1}): 2945.24-2643.8 (O-H str. of COOH), 1672.41(C=O str.), 1544.4 (C=C str. of phenyl ring), 1341 (S=O str. antisym of SO_2N), 1250.2 (S=O str. sym of SO_2N), 843.41 (out of plane C-H def due to p-subs in phenyl ring), 3382 (N-H str.), 1625 (N-H bending).^{1}H NMR (DMSO-d_6): δ 7.422 (d, 2H, 2', 6' H of C_6H_4-CH_3), 7.282 (d, 2H, 3', 5' H of C_6H_4-CH_3), 4.0 (t, 3H, -OC-C.H-CH_2-), 2.451(s, 1H, -CH_3), 2.2 (s, 1H, -SO_2NH-).^{13}C NMR (DMSO-d_6): δ 143.62 (C-1), 127.45 (C-2 & 6), 124.24 (C-3 & 5), 137.43 (C-4), 58.43 (C-7), 28.54 (C-8), 32.76 (C-9), 120.81 (C-11 & 15), 127.38 (C-12 & 14), 132.17 (C-13 & 19), 121.87 (C-17 & 21), 130.14 (C-18 & 20), 20.84 (C-22& 23), 21.76 (C-24). MS (FAB; *m/z*): 479. [M^{+1}]. Elemental analysis ($C_{26}H_{29}N_3O_4S$); calcd. C, 65.13; H, 6.05; N, 8.76; found C, 65.22; H, 6.21; N, 8.63%.

2-(4-Methylbenzenesulphonamido)-N,N′-bis(2-chlorophenyl)pentanediamide (5j)

White colour solid; yield: 88.21%; m.p.: 210-212°C; R_f 0.91; IR (KBr) υ_{max} (cm^{-1}): 2928.21-2571.22 (O-H str. of COOH), 1672 (C=O str.), 1532.14 (C=C str. of phenyl ring), 1322.07 (S=O str. antisym of SO_2N), 1213.02 (S=O str. sym of SO_2N), 850.2 (out of plane C-H def due to p-subs in phenyl ring), 3299.32 (N-H str.), 1630 (N-H bending).^{1}H NMR (400 MHz, DMSO-d_6): δ 7.4411 (s, 1H, 6' H of C_6H_4-CH_3), 7.221 (d, 2H, 3', 5' H of C_6H_4-CH_3), 7.241 (d, 2H, 2', 6' H of C_6H_4-Cl), 7.214 (d, 2H, 3', 5' H of C_6H_4-Cl), 4.02 (t, 3H, -OC-C.H-CH_2-), 2.382 (s, 1H, -CH_3), 2.33 (q, 4H,-H_2C-H_2C-), 2.120 (s, 1H, -SO_2NH-).^{13}C NMR (DMSO-d_6): δ 142.21 (C-1), 129.02 (C-2 & 6), 125.21 (C-3 & 5), 136.22 (C-4), 55.31 (C-7), 27.52 (C-8), 35.08 (C-9), 121.02 (C-11 & 15), 128.71 (C-12 & 14), 120.21 (C-17 & 21), 128.33 (C-18 & 20), 22.08 (C-22). MS (FAB; *m/z*): 520. [M^{+1}]. Elemental analysis ($C_{24}H_{23}N_3O_4SCl_2$); calcd. C, 55.38; H, 4.42; N, 8.07; found C, 55.21; H, 4.42; N, 8.09%.

2-(4-methylbenzenesulphonamido)-N,N′-bis(2-bromophenyl)pentanediamide (5k)

White colour solid; yield: 60.21%; m.p.: 199-201°C; R_f

0.80; IR (KBr) υ_{max} (cm^{-1}): 3011.45-2723.66 (O-H str. of COOH), 1578.21 (C=O str.), 1542.21 (C=C str. of phenyl ring), 1350.5 (S=O str. antisym of SO_2N), 1252.84 (S=O str. sym of SO_2N), 862.11 (out of plane C-H def due to p -subs in phenyl ring), 3289.35 (N-H str.), 1618 (N-H bending).^{1}H NMR (400 MHz, DMSO-d_6): δ 7.720 (d, 2H, 2', 6' H of C_6H_4-CH_3), 7.421 (d, 2H, 3', 5' H of C_6H_4-CH_3), 7.821 (s, 1H, 6' H of C_6H_4-Br), 7.971 (d, 2H, 3', 5' H of C_6H_4-Br), 3.42 (t, 3H, -OC-C.H-CH_2-), 2.401 (s, 1H, -CH_3), 2.11 (s, 1H, -SO_2NH-).^{13}C NMR (DMSO-d_6): δ 141.05 (C-1), 130.55 (C-2 & 6), 127.14 (C-3 & 5), 137.01 (C -4), 55.23 (C-7), 27.85 (C-8), 35.02 (C-9), 120.32 (C-11 & 15), 129.85 (C-12 & 14), 121.03 (C-17 & 21), 130.22 (C-18 & 20), 21.24 (C-22). MS (FAB; *m/z*): 609.[M^{+1}]. Elemental analysis ($C_{24}H_{23}N_3O_4SBr_2$); calcd. C, 47.29; H, 3.77; N, 6.89; found C, 47.07; H, 3.61; N, 6.81%.

***2-(4-Methylbenzenesulphonamido)-N,N'-bis(2-nitrophenyl)pentanediamide* (51)**

White colour solid; yield: 69.33%; m.p.: 215-217°C; R_f 0.83; Yield 69.33%, mp: 215-217°C. R_f 0.83. IR (KBr) υ_{max} (cm^{-1}): 2989.11-2628.86 (O-H str. of COOH), 1625.7 (C=O str.), 1570.24 (C=C str. of phenyl ring), 1313.02 (S=O str. antisym of SO_2N), 1211.09 (S=O str. sym of SO_2N), 870.57 (out of plane C-H def due to p-subs in phenyl ring), 3363 (N-H str.), 1633.2 (N-H bending).^{1}H NMR (400 MHz, DMSO-d_6): δ 7.706 (d, 2H, 2', 6' H of C_6H_4-CH_3), 7.521 (d, 2H, 3', 5' H of C_6H_4-CH_3), 7.811 (s, 1H, 6' H of C_6H_4-NO_2), 7.821 (d, 2H, 3', 5' H of C_6H_4-NO_2), 3.71 (t, 3H, -OC-C.H-CH_2-), 2.421 (s, 1H, -CH_3), 2.13 (s, 1H, -SO_2NH-).^{13}C NMR (DMSO-d_6): δ 139.29 (C-1), 126.51 (C-2 & 6), 129.24 (C-3 & 5), 136.82 (C-4), 59.08 (C-7), 28.07 (C-8), 39.22 (C-9), 121.02 (C-11 & 15), 129.52 (C-12 & 14), 124.28 (C-17 & 21), 128.75 (C-18 & 20), 21.22 (C-22). MS (FAB; *m/z*): 541. [M^{+1}]. Elemental analysis ($C_{24}H_{23}N_5O_8S$); calcd. C, 53.23; H, 4.25; N, 12.93; found C, 53.14; H, 4.30; N, 12.79%.

In vitro anti-cancer assay

Human breast cancer (MCF-7), leukemia (K-562), ovarian cancer (OVCAR-3), human colon adenocarcinoma (HT-29) and human kidney carci-noma (A-498) tumor cells were obtained from National Centre for Cell Sciences (Pune, India). The cultures were maintained in Dulbecco's Modified Eagles Medium (DMEM) containing 10% heat inactivated fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 µg/mL) at 37°C in 5%CO_2. Cells were grown in 25 cm^2 tissue cultures flask until confluent and used for cytotoxicity assays.

MTT assay

The MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide) assay (Mosmann, 1983) was modified and used to determine the inhibitory effects of test compounds on cell growth *in vitro*. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat bottomed tissue culture plate at a

Table I

***In vitro* anti-cancer activity (IC_{50}) of synthesized compounds against human breast cancer (MCF-7), leukemia (K-562), ovarian cancer (OVCAR-3), human colon adenocarcinoma (HT-29) and human kidney carcinoma (A-498)**

Com-pound	Human breast cancer (MCF-7)	Leukemia (K-562) CCRF-CEM HL-60(TB) K-562	Ovarian cancer (OVCAR-3)	Human colon adenocarcinoma (HT-29)	Human kid-ney carcinoma (A-498)
3	14.7 ± 0.0[a]	13.2 ± 0.0[a]	13.4 ± 0.0[a]	26.4 ± 0.0[a]	24.5 ± 0.0[a]
5a	11.5 ± 0.0[a]	10.3 ± 0.0[a]	21.3 ± 0.1[a]	37.9 ± 0.0[a]	27.3 ± 0.0[a]
5b	20.1 ± 0.1[a]	30.2 ± 0.0[a]	17.2 ± 0.0[a]	40.2 ± 0.0[a]	22.3 ± 0.0[a]
5c	75.2 ± 0.0	86.3 ± 0.0	97.2 ± 0.0	>100	87.5 ± 0.0
5d	62.3 ± 0.0[a]	88.3 ± 0.0[a]	89.7 ± 0.0[a]	78.3 ± 0.0[a]	77.3 ± 0.1[a]
5e	87.2 ± 0.0	89.3 ± 0.0	72.4 ± 0.0	>100	51.2 ± 0.0
5f	42.4 ± 0.0[a]	87.2 ± 0.1[a]	89.3 ± 0.0[a]	91.2 ± 0.0[a]	75.2 ± 0.0[a]
5g	87.3 ± 0.0[a]	70.3 ± 0.0[a]	82.3 ± 0.1[a]	88.9 ± 0.0[a]	56.4 ± 0.1[a]
5h	23.7 ± 0.0[a]	13.6 ± 0.0[a]	33.4 ± 0.0[a]	49.3 ± 0.1[a]	17.3 ± 0.1[a]
5i	22.3 ± 0.0[a]	32.2 ± 0.0[a]	27.2 ± 0.1[a]	40.1 ± 0.0[a]	19.5 ± 0.0[a]
5j	95.3 ± 0.0	74.3 ± 0.0	68.2 ± 0.0	87.2 ± 0.0	>100
5k	20.3 ± 0.0[a]	17.6 ± 0.0[a]	29.7 ± 0.0[a]	38.6 ± 0.0[a]	26.3 ± 0.0[a]
5l	87.2 ± 0.0[a]	75.6 ± 0.0[a]	56.2 ± 0.0[a]	77.9 ± 0.0[a]	30.3 ± 0.0[a]
Tamoxi-fen	8.2 ± 0.0[a]	10.4 ± 0.0[a]	9.3 ± 0.0[a]	14.3 ± 0.0[a]	12.2 ± 0.0[a]

Compounds with IC_{50} >100 µM were considered not active; [a]p<0.05 compared to tamoxifen (standard)

density of 5x10^3 cells/well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 48 hours incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of both standard (tamoxifen) and test compounds **5a-l** (8, 16, 32, 64, 128 and 256 µg/mL) in triplicates to achieve a final volume of 100 µL and then cultured for 48 hours. The compound was prepared as 1.0 mg/mL concentration stock solutions in PBS. Culture medium and solvent are used as controls. Each well then received 5 µL of fresh MTT (0.5 mg/mL in PBS) followed by incubation for 2 hours at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µL of DMSO to solubilize the colored formazan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 570 nm on an ELISA reader, Anthos 2020 spectrophotometer. Both standard and test maintained in triplicate. The IC_{50} value refers to the drug concen-tration that produces a 50% reduction in cellular growth when compared to untreated control cells (Holbeck, 2004).

In vivo anti-cancer assay

The animal experiments were performed following the approval of study protocols by the Institutional Animal Ethics Committee (BCRCP/IAEC/7/2012). The synthesized compounds were biologically screened against *Ehrlich ascites carcinoma* (EAC) in female Swiss Albino mice using tumor weight and cell count as activity parameters. Amongst various evaluation systems *in vogue*, this method has been standardized and numbers of screening results have been reported earlier (De and Pal, 1975; De and Pal, 1977; Ray and De, 2009). Two groups of Swiss Albino mice, each containing five healthy mice of the same sex (female in this case), approximately of same age and body weight (18-20 g), were selected at random and kept in two different cages under identical condition. One of these two groups served as control while the other as test. *Ehrlich ascites carcinoma* (EAC) cells were collected from the donor mice and were suspended in sterile isotonic solution (0.9% w/v NaCl). The numbers of tumor cells per ml of this suspension were counted under microscope with the help of haemocytometer. A definite number (about 2 x 10^6/0.2 mL) of these living viable cells was injected or implanted into the peritoneal cavity of each mouse. In this instance, the tumor cells multiplied relatively freely with in the peritoneal cavity and ascites developed. A day of incubation was allowed to establish the disease in the body before the administration of synthesized compounds. From the second day of transplantation up to the eight day a suitable dose (0.2 mmol/kg body weight) of the drug solution/suspension in sterile phosphate buffer (pH 7.2) was injected intraperitoneally to each mouse in the test group at 24 hours interval. Thus, seven doses of the drug were administered to each mouse in the test group. On the ninth day food and water were withheld or withdrawn 6 hours before the testing operation

Table II

***In vivo* anti-cancer activity of synthesized compounds against Ehrlich ascites carcinoma**

Compound	n	Ascitic cell (x 10^6 cells/mL)		%Inhibition	Weight of ascetic fluid (g)		%Inhibition
		Control	Test		Control	Test	
3	5	72.5	7.1 ± 0.0[a]	90.3	2.8	0.7 ± 0.1[a]	76.2
5a			0.8 ± 0.0[a]	98.9		0.2 ± 0.0[a]	93.0
5b			0.9 ± 0.0[a]	98.8		0.2 ± 0.1[a]	91.6
5c			16.0 ± 0.1[a]	78.0		2.3 ± 0.0[a]	19.8
5d			2.2 ± 0.0[a]	97.0		1.2 ± 0.0[a]	56.5
5e			1.44 ± 0.0[a]	98.0		2.0 ± 0.0[a]	27.9
5f			11.2 ± 0.0[a]	84.6		1.6 ± 0.1[a]	42.4
5g			1.3 ± 0.0[a]	98.2		0.3 ± 0.0[a]	88.5
5h			0.9 ± 0.0[a]	98.7		0.2 ± 0.0[a]	91.5
5i			1.0 ± 0.1[a]	98.6		1.0 ± 0.1[a]	65.2
5j			11.0 ± 0.1[a]	84.8		1.0 ± 0.0[a]	63.8
5k			1.0 ± 0.0[a]	98.6		0.1 ± 0.1[a]	96.4
5l			9.2 ± 0.0[a]	87.3		1.0 ± 0.0[a]	66.2
Mitomycin-C			0.0	100.0		0.00	100.0

[a]p<0.05 compared to mitomycin-C (standard)

started. The weight of all the animals was recorded before they were sacrificed. The peritoneal cavity was dissected and the ascites fluid was drawn by a syringe to a suitable volume with sterile ice cold saline and preserved in ice bath. The total number of living cells per milliliter in the peritoneal fluid of the three mice in a group was calculated. The fluid was sucked by absorbent cotton. The weight of the five mice after sacrifice was recorded. The evaluation of the test drug was made by comparing the cell count and ascetic fluid weight of the test with that of the control. The percentage inhibition of ascetic cell count and ascetic fluid weight was obtained by the following expressions.

Percentage inhibition of ascitic cell = (1 - T/C) x 100

Percentage inhibition of ascitic fluid = (1 - T′/C′) x 100

Where, T= Average number of ascitic cells per mL in test animals, C= Average number of ascitic cells per mL in control animals, T′= Average weight of ascitic fluid in test animals and C′= Average weight of ascitic fluid in control animals. Mitomycin-C (1 mg/kg body weight) in sterile phosphate buffer (pH 7.2) was used as standard.

Statistical analysis

Values are expressed as mean ± SEM and data was analyzed by ANOVA followed by Dunnet's test. $p<0.05$ was considered as significant.

Results and Discussion

The synthetic strategies followed for the preparation of the substituted 2-(4-methylbenzenesulfo-namido)pentanedioic acid bis amide derivatives **5a-l** are depicted in Scheme 1. Synthesis was started with chlorosulphonation of toluene, to get 4-methylbenzene-1-sulfonyl chloride **2**. This sulphonyl halide proved to be versatile synthon in the subsequent step in the preparation of substituted sulphonyl glutamic acids. With the application of Schotton-Bauman reaction, substituted sulphonyl glutamic acids **3** were prepared by one-step condensation of 4-methylbenzene-1-sulfonyl chloride **2** with L-glutamic acid. In this reaction alkaline medium was maintained to remove the hydrochloric acid which was formed during condensation. Reaction of the resulting intermediates with thionyl chloride afforded corresponding acid chloride followed by the amination with various amines afford the correspond-ding amines **5a-l**.

Formation of 2-(4-methylbenzenesulphonamido)pentanedioic acid bis amide derivatives **5a-l** were confirmed by recording their IR, NMR, mass spectra and elemental analyses. The IR spectra of compounds **5a-l** revealed the presence of absorption bands from 3012.54 to 2542 cm^{-1} that indicate the presence of OH str. of COOH, from 1696.07 to 1570.8 cm^{-1} for C=O str., from 1507 to 1577.31 cm^{-1} for C=C str. of phenyl ring, from 3287.32 to 3397 cm^{-1} for N-H str., from 1593-1645 cm^{-1} for N-H bending, from 1280.84-1354.5 cm^{-1} for S=O str. anti-symmetric of SO_2N and 1209-1310.18 cm^{-1} for S=O str. symmetric of SO_2N vibrations. In addition to proton signals of the functional groups and both aromatic ring present in the respective molecule ^1H NMR spectra of these compounds contained one proton singlet from δ 2.0 to 2.203 ppm which was assigned to -SO_2NH- proton and from 2.21 to 2.604 ppm for -CH_3 proton. The ^1H NMR spectra of compounds **5a-l** showed doublets from δ 7.201 to 7.98 ppm for aromatic protons and triplets from δ 3.54 to 4.1 ppm for -OC-CH-CH_2- proton confirming the formation of compounds **5a-l**. The ^{13}C NMR spectra of compounds **5a-l** showed peaks from δ 118.57 ppm to 156.38 ppm for aromatic protons, from δ 20.12 ppm to 22.31 ppm for -CH_3 carbon confirming the formation of compounds **5a-l**. The mass spectra of compounds **5a-l** showed molecular ion peaks [M^{+1}] at *m/z* corresponding to their respective molecular masses, which is in consistency with their respective molecular formulas. For the compound **5a**, molecular weight 385 is consistent with the molecular formula $C_9H_{19}N_5O_6S$. The values for the remaining compounds have been presented under the experimental part.

The cytotoxicity of the synthesized compounds **5a-l** and the intermediate compound number **3** were studied using the MTT assay in five human cancer cell lines, including human breast cancer (MCF-7), leukemia (K-562), ovarian cancer (OVCAR-3), human colon adenocarci-noma (HT-29) and human kidney carcinoma (A-498) (Table I). Compounds 2-(4-methylbenzenesulphonamido)-N,N′-bis(3-chlorophenyl) pentanediamide **5c**, 2-(4-methylbenzene-sulphonamido) -N,N′-bis(3-bromophenyl)pentane-diamide **5d**, 2-(4-methyl-benzene-sulphonamido)-N,N′-bis(4-bromophenyl)pentanediamide **5g** and 2-(4-methyl-benzenesulphonamido)-N,N′-bis(2-chlorophenyl) pentanediamide **5j** showed low cytotoxic effects on all the cell lines. Compounds 2-(4- methyl-phenylsulfonamido)pentanedioic acid **3**, N^1,N^5-dicarba-moyl-2-(4-methylphenylsulfonamido)pentanediamide **5a**, 2-(4-methylbenzenesulphonamido)-N,N′-(diphenyl) pentanediamide **5b**, 2-(4-methylben-zenesulphonamido)-N,N′-bis(4-nitrophenyl)pentanediamide **5h**, 2-(4-methylbenzenesulphona-mido)-N,N′-bis(4-nitrophenyl) pentanediamide **5i** and 2-(4-methylbenzenesulphonamido)-N,N′-bis(2-bromophenyl)pentanediamide **5k** showed high cytotoxicity in all cell lines with IC_{50} concentrations lines, except for the human colon adeno carcinoma (HT-29) cell line. The primary antitumor activity of tamoxifen by inhibition protein kinase C (Gelman, 1997) and also ability to facilitate the apoptosis in cancer cell not expressing estrogen receptor is due to generation of oxidative stress resulting in thiol depletion and activation of the transcriptional

factor NF-kappaB. Many clinical studies explain the tamoxifen application in various kinds of maligamant diseases (Ferlini et al., 1999; NCI, 1999).

All the newly synthesized final compounds **5a-l** along with compound no. **3** were screened for their anti-cancer activity against *Ehrlich ascites carcinoma* is summarized in Table II together with mitomycin-C. Among the synthesized com-pounds, the ureide **5a**, anilide **5b**, *p*-nitoanilide **5h** and *o*-bromoanilide **5k** derivatives showed encouraging activity in both the parameter, *viz.*, ascetic fluid weight (93.0% for **5a**, 91.6% for **5b**, 91.5% for **5h** and 96.4% for **5k**) and ascetic cell count (98.9% for **5a**, 98.8% for **5b**, 98.7% for **5h** and 98.6% for **5k**) respectively. Hence, a detailed and prolonged study is necessary to establish their activity in other models.

Thus, twelve new compounds along with an interme-diate compound **3** were synthesized, characterized and biologically screened for *in vitro* and *in vivo* antitumor activity. It was noticed that final derivative compounds showing better activity than the parent compound and it may be due to the substituents present in those compounds. These observations encourage performing QSAR study in future.

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Conflict of Interest

Authors declare no conflict of interest

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